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IMPERIAL BUREAU OF MYCOLOGY
13 JUL. 1921

BULLETIN No. 220

BLACK SPOT OF ONION SETS

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URBANA, ILLINOIS, MAY, 1919

Contents of Bulletin No. 220

PAGE

Introduction 507

The Disease 507

The Three Types 508

The Causal Fungus 509

Culture Studies 509

The Subicle 510

The Tubercle 510

The Perithecium 511

Correlation with Earlier Morphological Work 529

Taxonomic Position of the Ascigerous Stage 529

Suggestions Regarding Control 531

Bibliography 532

BLACK SPOT OF ONION SETS

VOLUTELLA CIRCINANS AND ITS PERITHECIAL FORM, CLEISTOTHECOPSIS CIRCINANS

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INTRODUCTION

Serious losses of onions during storage, particularly of onion sets, led to the present study. Loss in storage, especially of the white varieties, is very high. It is estimated that in the Chicago district alone, for the 1917 season, there was a loss of approximately 150,000 bushels of sets valued at about $300,000.

In the present study onions and onion sets were examined either directly in the markets and stores or by sample lots which were supplied by large holders. Results of such examinations showed the presence of several fungi apparently as the cause of loss in storage, as follows: "Vermicularia," Sclerotium, Botrytis (several species), Fusarium, Aspergillus, Bacillus, and a few apparently saprophytic forms. The so-called "Vermicularia" was present in 60 to 80 percent of the white onion sets examined, and Sclerotium in 2 to 7 percent. The other fungi appeared in a lesser quantity. It is to the fungus found most generally present that chief attention has been given in the studies reported in this bulletin.

THE DISEASE

This disease is easily recognized by both macroscopic and microscopic observation. As a disease it was first described under the name "*Vermicularia circinans*" by Berkeley in *Gardener's Chronicle* in 1851.[1a] It has later been referred to by Clinton,[2] Cooke,[3] Halsted,[4] Massee,[5] Selby,[6] Stevens,[7] Stevens and Hall,[8] Stoneman,[9] Thaxter,[10] Tubeuf and Smith,[11] Voglino,[12] and a few others. It has been reported under many common names, such as anthracnose, rot, smudge, surface rot of the bulb, mold, vermiculariose, black spot, and neck rot. The records of the Plant Disease Survey, U. S. Department of Agriculture, show its distribution in the following states: Connecticut, Indiana, Louisiana, Maryland, Massachusetts, Minnesota, New York, Ohio, Pennsylvania, Texas, and Wisconsin.

[a]Superior figures are used thruout this bulletin to indicate the literature citations given in the bibliography, page 532.

THE THREE TYPES

The disease assumes three distinctly different aspects, which are here described separately as Types A, B, and C.

Type A.—This type of the disease is the most common and the most striking in appearance. It is the one that is usually regarded as typical and the one commonly described and figured as the onion disease due to "Vermicularia." Upon the dry outer scale of the bulb it presents an approximately circular spot, usually about the size of a dime or a quarter. The hue is prevailingly black, owing to much black mycelium within the tissues, and numerous black knots of mycelium are seen, often grouped in a series of concentric circles, as shown in Fig. 1 (1). (See also Massee,[5] Fig. 130, and Thaxter,[10] Fig. 44.)

Variations from this typical concentric arrangement are met. In some instances the prevailing direction of the mycelium is at right angles to the main veins, resulting in transverse black bands (see cover illustration). In other instances neither the concentric nor the banded arrangement predominates but the spots consist of irregularly arranged minute specks, as illustrated in Fig. 1(2). In still other instances the area involved in the spot is entirely black and the minute component black specks are lost to view, as in Fig. 1 (3, 4). (See also Clinton,[2] Plate 20, Fig. a). Another view of the condition shown in Fig. 1 (1) and in the cover illustration is shown in Fig. 2 (1). In many of these spots there is a pronounced tendency for the fungus to follow the course of the veins, as may be seen in Fig. 2 (2). All of the varying forms of Type A occur on the outermost scales of the bulb, as in Fig. 2 (3), and in all cases on scales which are perfectly dry.

Type B.—This type, like Type A, occurs on the dry outer scales but it is usually limited to the upper part of the bulb, as illustrated in Fig. 3 (1); at least it always starts at the top of the bulb and progresses toward the base. This type may assume any of the variations mentioned under Type A. Several views are shown in Figs. 4 and 5. The essentially distinguishing character of this type is that the invasion of the fungus starts at the top of the bulb, usually stopping with the drying of the scale before the equatorial region is reached. It is probable that Type B results from infection thru the wounds caused in removing the "top."

Type C.—This type is manifest in those scales of the bulb which are still succulent and juicy or which remained so for considerable time after the invasion by the fungus. As shown in Fig. 3 (2), the spots may lie directly under and originate from spots of Type A, but on account of their very different appearance and character this type is described separately. Fig. 3 (3) shows Type A; Fig. 3

(4) shows the same bulb as Fig. 3 (3) but with the outer scale removed, showing Type C below. In Type C the fungus, owing to the fact that it lives and develops for a longer time within the juicy tissue, attains developmental stages not shown in the more limited life period in the outer scales. The chief manifestation of this development is in the formation of tubercles, or warty growths. These are usually located at the centers of the infected areas, whether the spot is small, 2 to 3 mm., or larger, .5 to 1 cm. In the older, larger spots, secondary or tertiary series of tubercles may occur, often in roughly concentric arrangement. The tubercles are frequently 1 or even 2 mm. in diameter and rise fully 1 mm. above the surrounding scale. The distinguishing character of this type is the presence of these tubercles and the fact that the disease often remains progressive, tho of course scales with the tubercular development may later dry and thus inhibit further progress.

That these three types of disease are due to the same fungus is fully borne out by the study of the culture characters, by the microscopic studies detailed below, and by the fact that the types to an extent merge one into the other.

THE CAUSAL FUNGUS

The mycelium is rather coarse, 3.6 to 10.8 μ wide, branches frequently and irregularly, and is cut by septa at irregular intervals, usually about 12 to 16 μ apart. It is crooked, intercellular, and intracellular, or even superficial (Figs. 8, 9, 10). When young it is hyaline, later turning quite dark. The fungus shows a strong tendency to develop mats of parallel hyphae, as illustrated in Fig. 7 (3). It is these that are largely responsible for the banded appearance shown in the figure on the front cover. It is also characteristic of the mycelium to produce branches, the terminal cells of which turn dark and swell, producing an effect much like that of the so-called appressoria of the germinating anthracnose spores. Intercalary cells also often darken and swell, as do frequently whole series of cells.

CULTURE STUDIES

Studies of the fungus in agar showed general agreement with the facts stated above. The methods used in isolating the fungus were: direct planting on plates of small pieces of infected onion tissue; dilution plating of spores in agar; and direct planting of spores which had previously germinated. For most of the studies an onion-broth agar was used.

When white onions were used in the agar, the growth of fungus was abundant; when yellow and red onions were used, the growth was slight and not many sporing bodies developed. In Petri-dish

cultures the fungus produced a light-colored mycelium near the upper surface of the medium and under some conditions an aerial mycelium. Contrasting the structure with that found on the onion in nature, the cells of the mycelium were much more elongated and very seldom was there an appearance of oil drops in them. Moreover, on agar there were very few of the dark, swollen end cells so characteristic of the fungus under natural conditions. As the colony became older, the aerial mycelium gradually shriveled and the colony assumed a gray or grayish-brown color with numerous small protuberances on the surface. These are the sporodochia, acervulus-like structures on subicles of interwoven hyphae. They were not confined to the upper surface alone but often developed below the surface of the agar. Many of the sporodochia were seen to have a pedicel, or neck, arising from the subicle. As the fungus grew older, it often colored the medium a dark brown. A portion of an agar plate showing a colony is shown in Fig. 15. The small black dots seen are the sporodochia.

THE SUBICLE

As the fungus ages, the mycelium in numerous points develops subicles, which are at first one cell thick. Various stages are shown in Figs. 6 (1, 2, 3), 7 (3), and 12 (1). The subicle may attain dimensions of 224 to 784 μ by 336 to 1,344 μ. Often setae appear upon it, varying in number from one to many, as shown in Figs. 6 (1, 3) and 7 (3). Fig. 12 (1) shows one of these setigerous subicles in section. The setae are 125 to 240 μ long, about 4 μ wide at the base, acute at the apex, and very dark in color.

THE TUBERCLE

When the fungus grows for a sufficient time in the still juicy scale, numerous host cells as well as intercellular spaces become filled and largely distended with mycelium, as shown in Fig. 8 (1, 2, 3). This results in greatly increasing the thickness of the host at these points, producing distinctly swollen spots (Figs. 9 and 10). The subicles, present superficially over these regions, increase in thickness by division of the cells in a direction tangential to the surface of the onion, resulting in distinct rounded protuberances, or tubercles. Several stages in the development of these structures are shown in Fig. 11 (1, 2, 3, 4).

The Sporodochium.—This is seen to consist of a pseudoparenchymatous inner tissue covered by a continuous surface layer. It may or may not bear setae in the early stages of development. The young sporodochium eventually ruptures its covering membrane. Occasionally the surface is overgrown by loose mycelial masses, as shown in Fig. 12 (2).

When sufficient growth time allows, especially when one of the deeper scales of the onion is affected, the sporodochium increases largely in diameter, reaching even more than 2 mm., and becomes nearly flat-topped or corrugated, as shown in Figs. 13 (2), 14, and 16. In all cases the conidiophores are borne upon a raised superficial base which constitutes the sporodochium (see Figs. 13 (1, 2), 14, and 16), in contradistinction to the innate form of the acervulus, which has no such base. The tubercular swelling, due to the massing of mycelium below and in the epidermis, partakes of sporodochial character also, and while this subepidermal part may not be regarded as constituting a true sporodochium, it serves to emphasize the tendency of the fungus to produce such structures.

Conidiophores.—Soon after rupturing the covering membrane, the sporodochium develops on its surface numerous parallel conidiophores of palisade arrangement, as shown in Figs. 12 (3) and 13 (1, 2, 3). Interspersed with the conidiophores are few or many setae. The conidiophores are 24 to 48 μ long, 2.4 μ thick, straight, simple, hyaline, few-septate, obtuse, and bear the conidia acrogenously.

Conidia.—The conidia are falcate, acute at each end, continuous, hyaline, 19.2 to 26.4 μ by 3.6 to 7.2 μ. In germination they become one-septate and send out germ tubes from one or both cells, from either the side or the tip of the spore. Appressoria similar to those produced by Gloeosporium are also found in abundance.

THE PERITHECIUM

Onion sets which were heavily infected with the fungus under discussion were placed in sand in the greenhouse. Upon examination of certain of these sets after about thirty days, black bodies were seen on the outer scales. Some of these were imbedded for microtome sectioning; others were examined direct.

These structures, the perithecia, are irregularly globular, dark brown to black, and from 89.6 to 313.6 μ in extreme dimensions. The surface is characteristically reticulated, as seen in Fig. 17 (1), and there are often numerous short hairs growing from the surface cells. The perithecia are borne either in or on the host tissue. Organically connected with the perithecia is the typical mycelium of the Volutella, a point that is especially clear in the study of young perithecia. Fig. 17 (3). There is no ostiole. Microtome sections show the perithecia in early stages to resemble sclerotia in general structure, i. e., the whole structure is pseudoparenchymatous, the cells of the outer layer being darker and thicker-walled than those of the interior. This point is illustrated in Fig. 17 (1 and 4). In a later stage an ascogenous region is differentiated. This assumes the

form of numerous filiform hyphae of nearly parallel direction arising from the basal portion of the perithecium, as shown in Fig. 18 (2). Perithecia of this age when crushed release this ascogonium *en masse*. In still older perithecia the central part is filled with asci, lying in close contact and without paraphyses, as seen in Fig. 18 (3). In still later stages the asci disappear and the ascospores lie free in the perithecial cavity. Figs. 18 (4) and 19. In Fig. 17 (4) is shown a perithecium in a very early stage of development; general views of ascigerous perithecia are shown in Figs. 18 (3, 4) and 19. The asci are here seen to arise *approximately* from a region which may be designated as basal, i. e., not in Plectascinaceous fashion. The asci are about 72 to 96 μ by 19.2 to 24 μ and are evanescent, disappearing early.

The spores are dark, obtuse at each end, 24 to 36 μ by 9.6 to 14.4 μ, muriform, usually with 4 to 7 μ transverse and 1 to 2 μ longitudinal septa, as seen in the optical section shown in Fig. 17 (2). Two variant spores were found (*in situ* in microtome section), each with one septum, Fig. 17 (2). These would be regarded as immature except for the fact that they were of mature color. It is probable that the spores do really show this extreme variation in septation and that the septa may increase greatly in number even after maturity of the spores.

The evidence that these perithecia belong to the Volutella may be summarized as follows: (1) they occurred on sets badly infected with the Volutella; (2) no other fungi or other types of mycelium were seen to be connected with them; (3) when studied in various stages of development, the typical Volutella mycelium, which offers definite characters for recognition, was seen in organic connection with them, as illustrated in Fig. 18 (1); (4) the outgrowths from the perithecia are like those of the Volutella.

FIG. 1.—VOLUTELLA, TYPE A: (1) SHOWING CONCENTRIC ARRANGEMENT OF FUNGUS KNOTS; (2) BLACK SPECKS IRREGULARLY ARRANGED THRU THE SPOTS; (3) DIFFUSE, NEARLY STRUCTURELESS, BLACK BLOTCHES; (4) SAME AS (3)

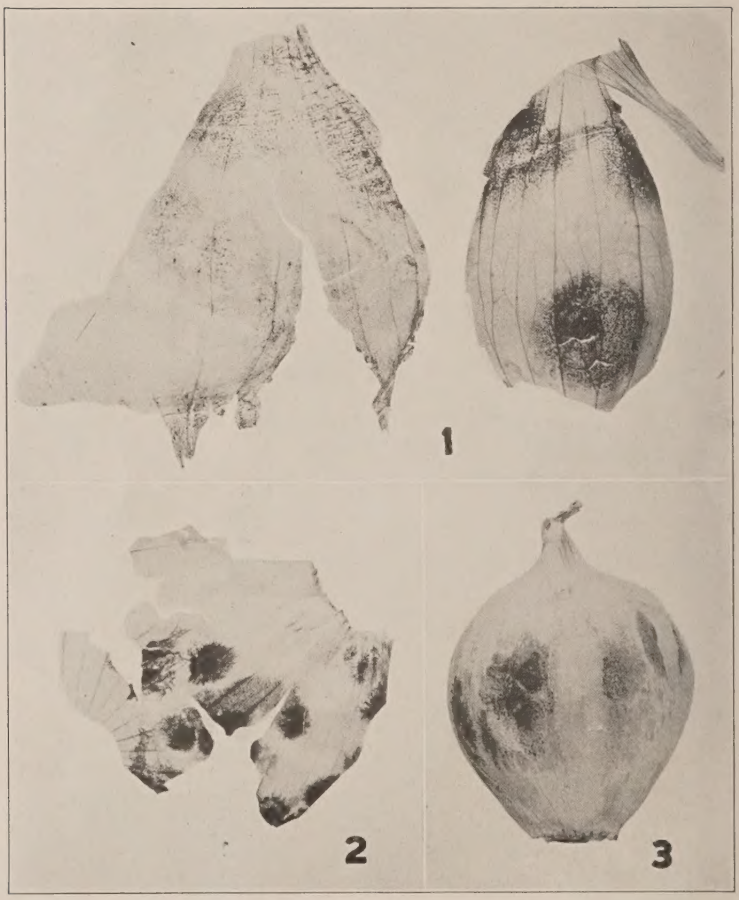

FIG. 2.—VOLUTELLA, TYPE A: (1) OTHER VIEWS OF PORTIONS SHOWN IN FIG. 1 (1) AND IN THE COVER ILLUSTRATION; (2) ILLUSTRATING A TENDENCY OF THE FUNGUS TO FOLLOW A VEIN; (3) A PORTION OF THE DRY OUTER SCALE HAS BEEN REMOVED SHOWING THE FLESHY SCALE BELOW TO BE CLEAN AND HEALTHY

FIG. 3.—VOLUTELLA: (1) TYPE B. THE SPOT IS LIMITED TO THE TOP OF THE BULB; (2) TYPE C. SPOT IN A LIVING SCALE BELOW A SPOT SUCH AS SHOWN IN FIG. 1 (1); (3) SHOWING CHARACTERISTIC TYPE A, ALSO TYPE C BENEATH; (4) TYPE C. SAME AS (3) BUT WITH THE OUTER SCALE REMOVED

FIG. 4.—VOLUTELLA, TYPE B. SHOWING VARIATIONS IN THE TYPE

FIG. 5.—VOLUTELLA, CHARACTERISTIC TYPE B

Fig. 6.—(1) A Subicle Bearing Several Setae, h.p.; (2) Subicle Similar but Without Setae, h.p.; (3) Subicle in Very Young Stage Bearing One Seta, h.p.

Fig. 7.—(1) Photograph of Tubercles with Setae as Seen in Side View; (2) A View of Setose Tubercle from Above; (3) Showing Mats of Parallel Hyphae Developed in Early Stages, Also Subicles of Comparatively Large Size, Some with Setae

FIG. 8.—(1) SUPERFICIAL MYCELIUM ON A DRIED SCALE, H.P.; (2, 3) INTERNAL MYCELIUM IN A DRIED SCALE, H.P.

Fig. 9.—Showing a Superficial Subicle Over a Vein, h.p.

Fig. 10.—Dried Scale, Volutella, Type A. A Section Showing That the Fungus Is Epidermal as Well as Subepidermal and Subcuticular. Showing Also the Relation of the Fungus to the Vein, h.p.

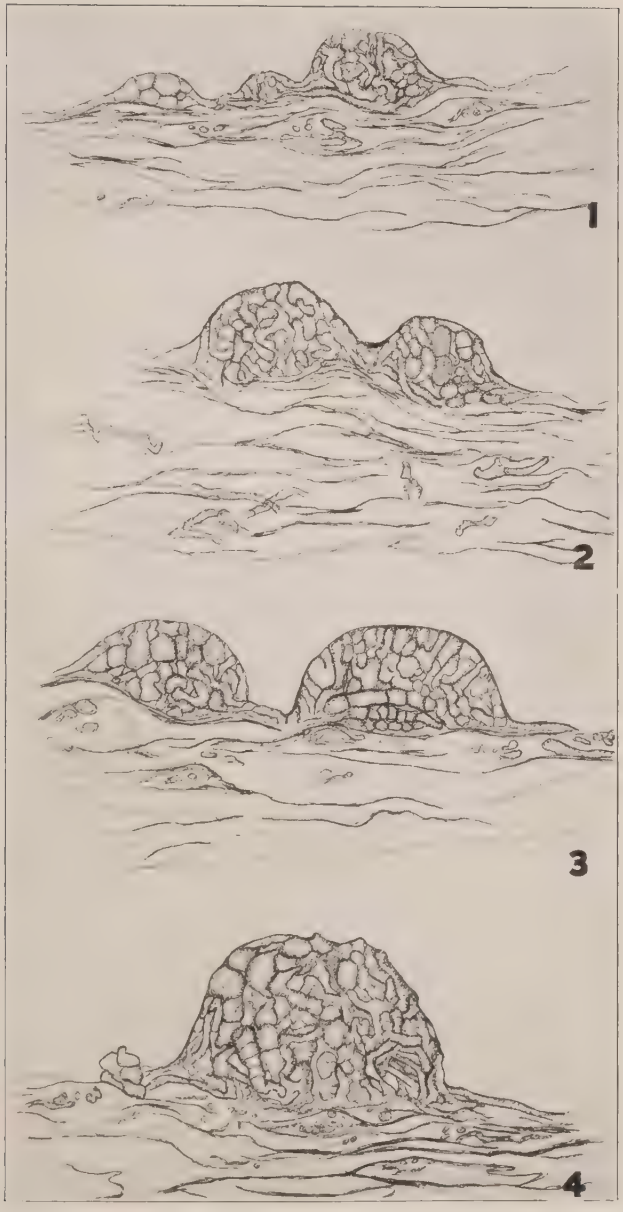

Fig. 11.—(1, 2, 3, 4) Early Stages in the Development of the Tubercle, H.P.

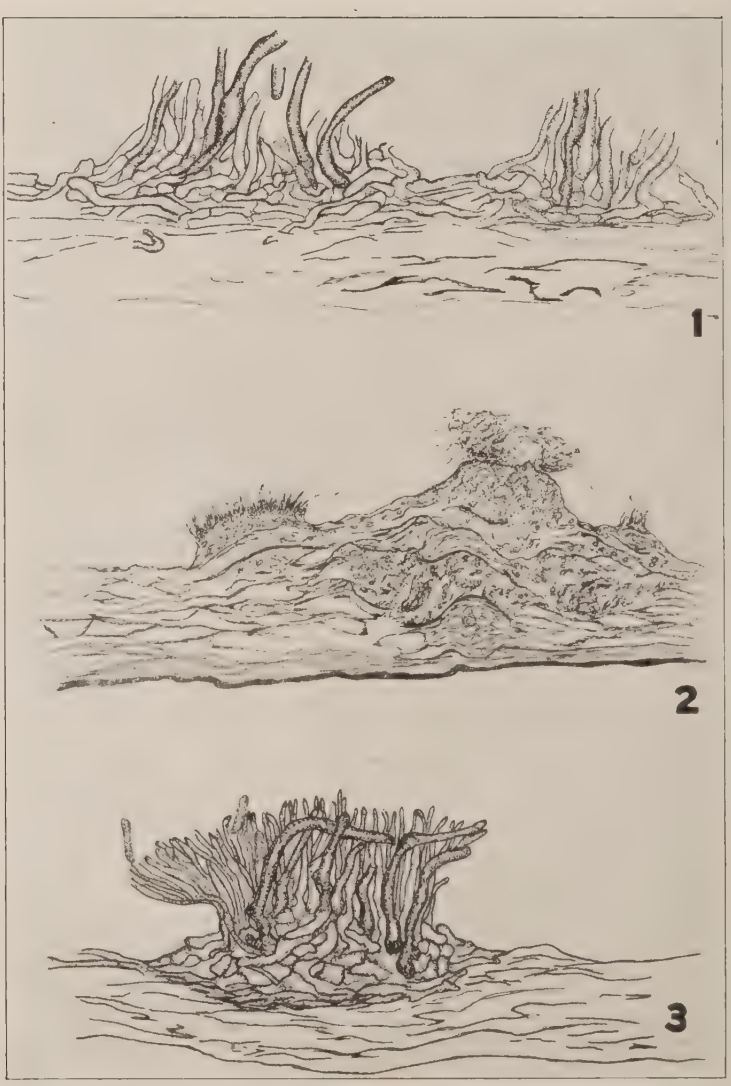

FIG. 12.—(1) A SETIGEROUS SUBICLE IN SECTION, H.P.; (2) VIEW TO SHOW THE THICKENING OF THE ONION SCALE BY THE FUNGUS, THE DEPOSITION OF MASSES OF FUNGUS MYCELIUM, AND THE RELATION TO THE SPORODOCHIUM, L.P.; (3) A SMALL SPORODOCHIUM WITH SETAE AND CONIDIOPHORES ON A DRIED ONION SCALE, H.P.

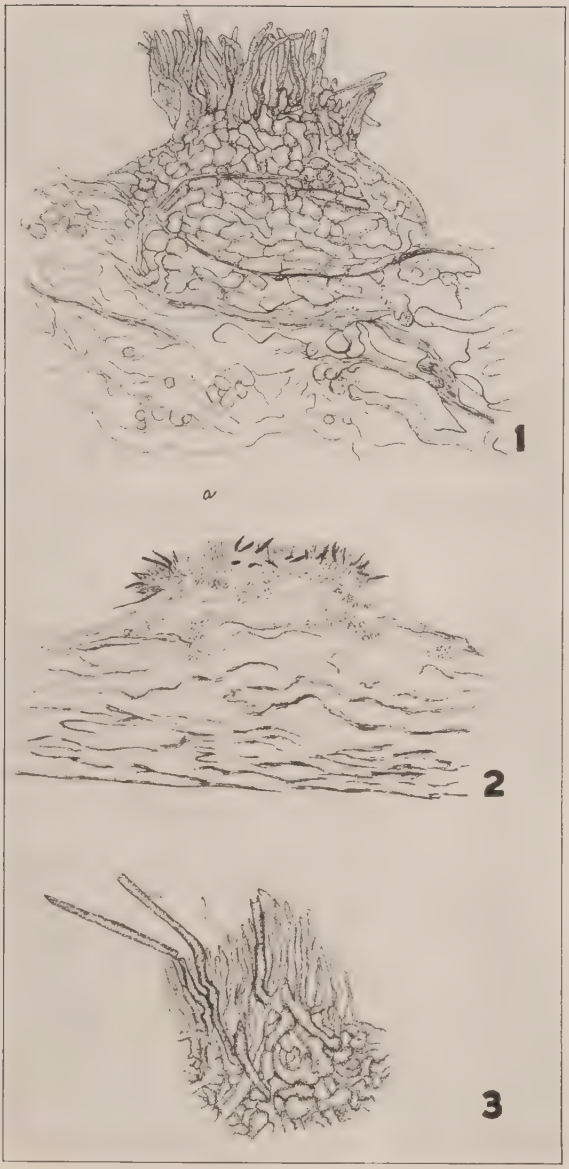

FIG. 13.—A TUBERCLE WHICH HAS RUPTURED AT THE TOP PREPARATORY TO BECOMING CONIDIIFEROUS AND SETIGEROUS, H.P.; (2, 3) MEDIAN SECTION THRU A SETIGEROUS, CONIDIIFEROUS SPORODOCHIUM. A DETAIL OF SETAE, CONIDIOPHORES, AND CONIDIA, H.P.

Fig. 14.—A Large Setigerous, Conidiiferous Sporodochium in Median Section, L.P.

Fig. 15.—Photograph of a Portion of an Agar Colony ×10

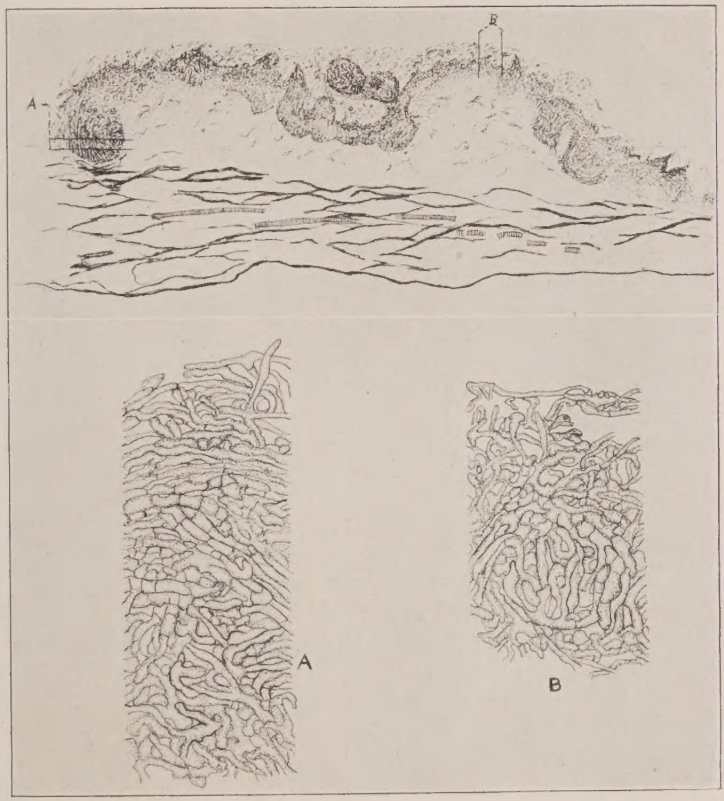

FIG. 16.—MEDIAN SECTION THRU AN OLD, LARGE SPORODOCHIUM. A LOW-POWER DRAWING SHOWING SHAPE AND RELATION TO THE SUBSTRATUM; THE PORTIONS A AND B ENLARGED TO SHOW CELLULAR STRUCTURE, H.P.

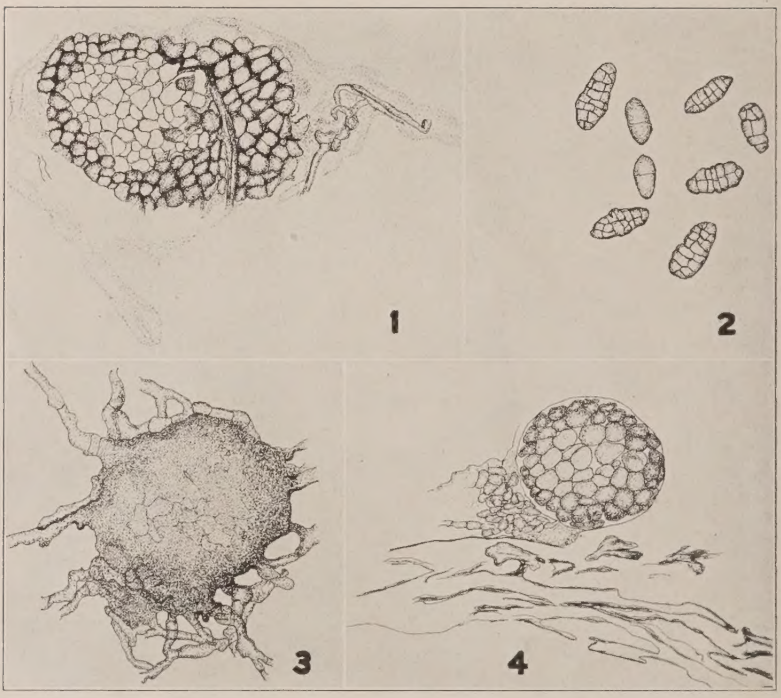

FIG. 17.—(1) A PERITHECIUM IN TANGENTIAL SECTION SHOWING SURFACE CELLS, ALSO CELLS JUST BELOW THE SURFACE, H.P.; (2) ASCOSPORES, H.P.; (3) A VERY YOUNG PERITHECIUM, H.P.; (4) A VERY YOUNG STAGE IN THE DEVELOPMENT OF A PERITHECIUM, MEDIAN SECTION SHOWING ATTACHMENT TO A BASAL STRUCTURE, H.P.

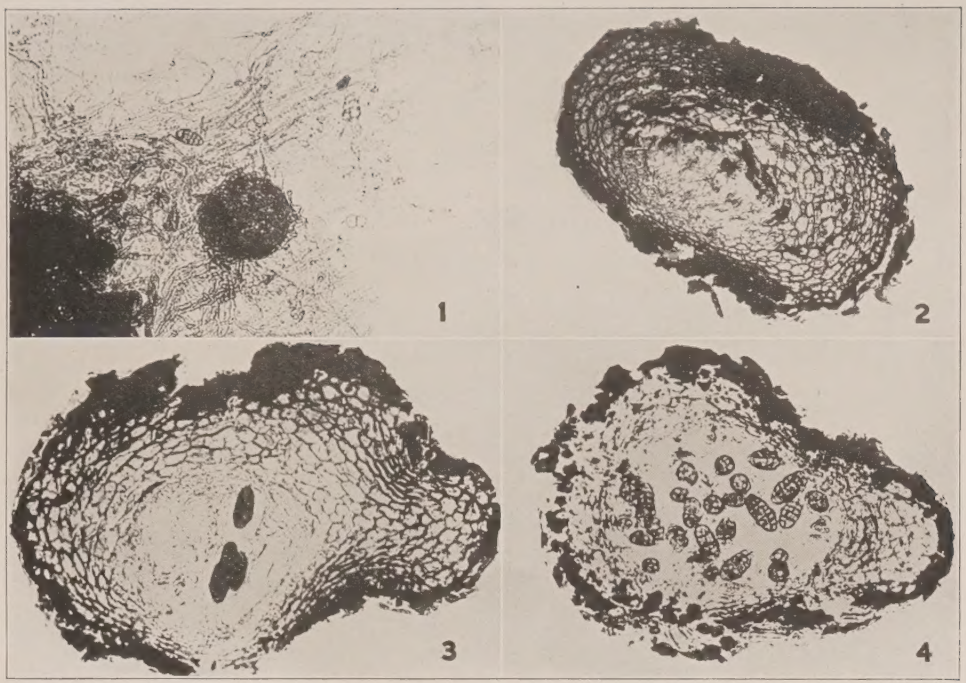

FIG. 18.—(1) A YOUNG PERITHECIUM SHOWING VOLUTELLA MYCELIUM CONNECTED WITH IT. NEARBY IS AN ASCOSPORE; (2) A PERITHECIUM IN WHICH AN ASCOGENOUS REGION IS DIFFERENTIATED; (3) A PERITHECIUM WITH MATURE SPORES IN THE ASCUS; (4) MATURE PERITHECIUM WITH ASCOSPORES FREE

Fig. 19.—Mature Perithecium in Median Section. Spores Free, h.p.